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Elevated serum levels of neutrophil elastase in patients with influenza virus-associated encephalopathy

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ABSTRACT

We examined serum levels of various cytokines, chemokines, growth factors, and adhesion molecules in patients with uncomplicated influenza (n = 20) and influenza virus-associated encephalopathy (IE) (n = 18) to understand the underlying mechanism of IE. We found that IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, G-CSF, GM-CSF, TNF-α, TIMP-1, MMP-9, sE-selectin, and neutrophil elastase were elevated significantly in sera from patients with uncomplicated influenza and those with IE, compared with normal controls (n = 20). Of note, neutrophil elastase, sE-selectin, IL-8, and IL-13 were elevated significantly in IE as compared with uncomplicated influenza. In the present study, for the first time, we found that serum levels of neutrophil elastase were increased in patients with IE compared with uncomplicated influenza, which suggested that cerebral endothelial damage in the development of IE was mediated by neutrophil elastase. The present study implied that anti-elastase agents are possibly an effective therapeutic protocol for IE, but this needs further elucidation.

1. Introduction

Influenza virus-associated encephalopathy (IE) is the most common encephalopathy in Japan [1,2]. Most patients with IE have severe neurological sequelae [1,2]. Despite extensive investigation, the pathogenesis of this condition remains elusive [3–5]. In some patients with acute encephalopathy, genetic predispositions, such as ADORA2A polymorphisms [6], fever-sensitive SNPs in the carnitine transporter II gene [7], or mutations in the Toll-like receptor 3 [8], SCN2A [9], and SCN1A genes [10], have been considered contributing factors in the development of acute encephalopathy.

Previous studies demonstrated that serum and cerebrospinal fluid concentrations of several proinflammatory cytokines and cytokine receptors, including interleukin (IL)-6, IL-1β, and soluble TNF receptor 1, are elevated and related to the clinical severity of IE [11–13]. Moreover, other cytokines and their receptors, cell adhesion molecules, and other factors are also related reportedly to the severity of IE. These include IL-10, sE-selectin, sCD163, sCD40L, cytochrome *c* in the serum [13–17], and d-ROM in the CSF [18]. These findings demonstrate that influenza virus-induced inflammation is a major contributor to IE.

However, the reasons why Asian children are prone to affliction with IE remain unknown. Understanding the pathophysiology of IE is very important for establishing an effective treatment strategy and prophylactic approach. We examined serum levels of various cytokines, chemokines, growth factors, and adhesion molecules in patients with uncomplicated influenza and IE to understand the underlying mechanism of IE.

2. Materials and methods

We collected serum samples from control patients with uncomplicated influenza and patients with IE. The serum samples were stored

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Table 1
Results of comparative analyses of cytokines, chemokines, growth factors, adhesion molecules, and neutrophil elastase between control patients, and patients with uncomplicated influenza, and influenza virus-associated encephalopathy.

Mean ± SE						
	Control (20)	Flu (20)	FluE (18)	p (control vs flu)	p (control vs fluE)	p (flu vs fluE)
IL-1β	2.34 (2.08)	0.99 (0.25)	8.18 (6.63)	***	*****	n.s.
IL-2	3.19 (1.72)	10.23 (3.5)	15.38 (4.91)	*	***	n.s.
IL-4	0 (0)	0.14 (0.14)	7.49 (6.55)	n.s.	n.s.	n.s.
IL-5	0.42 (0.09)	1.01 (0.17)	2.53 (1.41)	****	****	n.s.
IL-6	5.68 (1.58)	131.61 (23.48)	2720.82 (2085.81)	******	******	n.s.
IL-7	4.81 (0.48)	11.89 (0.74)	7.90 (1.03)	******	*	**
IL-8	17.86 (12.67)	15.11 (1.97)	380.59 (317.17)	*****	******	*
IL-10	2.55 (1.38)	13.83 (2.56)	75.12 (35.07)	******	******	n.s.
IL-12	0.68 (0.56)	0.01 (0.01)	0.73 (0.45)	n.s.	n.s.	n.s.
IL-13	0.57 (0.17)	1.83 (0.38)	2.72 (0.42)	***	******	*
IL-17	2.86 (1.44)	15.89 (13.20)	3.91 (1.26)	n.s.	n.s.	n.s.
G-CSF	0.87 (0.40)	29.0 (6.28)	913.41 (743.66)	******	******	n.s.
GM-CSF	1.52 (0.86)	32.86 (13.83)	40.62 (13.69)	*	***	n.s.
IFN-γ	157.61 (21.56)	151.66 (15.81)	195.1 (30.35)	n.s.	n.s.	n.s.
TNF-α	0.44 (0.34)	1.98 (0.43)	13.35 (9.65)	*****	*****	n.s.
MCP-1	71.59 (7.43)	460.93 (246.58)	788.92 (588.02)	*****	n.s.	n.s.
MIP-1β	157.61 (21.56)	151.66 (15.81)	196.0 (30.35)	n.s.	n.s.	n.s.
Elastase	123.5 (24.3)	243.3 (44.9)	544.6 (78.9)	*	******	****
TIMP-1	195.2 (22.2)	387.2 (135.6)	320.5 (37.9)	*	*	n.s.
VEGF	170.8 (33.5)	120.2 (18.5)	146.0 (33.7)	n.s.	n.s.	n.s.
MMP-9	89.0 (15.6)	330.7 (55.8)	406.0 (94.4)	***	******	n.s.
sE-selectin	67.8 (7.2)	81.2 (8.5)	174.4 (26.1)	n.s.	******	****
NSE	5.82 (0.77)	2.27 (0.5)	3.43 (0.86)	***	*	n.s.

n.s.: not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.
**** $p < 0.001$.
***** $p < 0.0005$.
****** $p < 0.0001$.

at less than −20 °C until examination. Serum samples from 18 patients with IE, ranging in age from 8 months to 9 years (median 2.3 years), were analyzed. The diagnosis of IE was based on disturbed consciousness that lasted more than 24 h and a convulsive status. Abnormal brain CT and MRI results were also included in the diagnosis. Blood was drawn on the day of admission to the local hospital or Tohoku University Hospital after receiving informed consent from the family. Therefore, the samples examined reflect an early phase of encephalopathy. Two patients died of multiple organ failure, and eight patients had neurological sequelae, which consisted of spastic quadriplegia, visual disturbance, and epilepsy, as well as higher brain dysfunction, including aphasia and learning disabilities. Eight patients had no neurological sequelae.

As an age-matched control group, 20 serum samples drawn for routine analysis from patients (6 months–10 years; median 3.8 years) with non-inflammatory disease and 20 serum samples from patients with uncomplicated influenza (9 months–7 years; median 3.3 years) were obtained after receiving informed consent. The diagnosis of influenza was performed using a quick ELISA test for influenza A and influenza B. All 20 samples from patients with uncomplicated influenza were drawn on the first visit to the hospital after experiencing fever. Therefore, samples reflect the early changes in serum levels associated with influenza virus infection. These patients did not have any signs of encephalopathy or febrile convulsions.

The study design and purpose were approved by the institutional review board of Tohoku University and explained fully to all patients and/or their guardians. Informed consent was obtained from all patients.

The concentrations of 17 cytokines/chemokines, including interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 IL-13, and IL-17, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein (MIP)-1β, were measured in the sera of all patients and controls using a multiplex bead-based assay (Bio-Plex Multiplex Suspension Array System, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Briefly, serum samples were diluted 1:4 and incubated with antibody-coupled beads. The complexes were washed and incubated with the biotinylated detection antibody followed by streptavidin–phycoerythrin, after which the cytokine concentrations were measured. Standards ranging from less than 10 pg/mL to more than 5050 pg/mL were used to generate broad-range standard curves.

We also measured serum levels of soluble E-selectin (sE-selectin), neuron specific enolase (NSE), neutrophil elastase, tissue inhibitor of metalloproteinase-1 (TIMP-1), matrix metalloproteinase-9 (MMP-9), and vascular endothelial growth factor (VEGF) by ELISA; materials for these tests were purchased from Bender MedSystems (sE-selectin), Biomeda (NSE), Abcam (neutrophil elastase), and Amersham Bioscience (TIMP-1, MMP-9, VEGF).

The statistical analysis was performed using the Mann–Whitney *U*-test, with significance set at $p < 0.05$.

3. Results (Table 1, Figs. 1 and 2)

We found that IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, G-CSF, GM-CSF, TNF-α, TIMP-1, MMP-9, sE-selectin, and neutrophil elastase were elevated significantly in sera from both patients with uncomplicated influenza and those with IE compared with normal controls. Of note, neutrophil elastase, sE-selectin, IL-8, and IL-13 were elevated significantly in IE compared with uncomplicated influenza. On the other hand, NSE was decreased significantly in both sera from patients with uncomplicated influenza and those with IE, compared with the normal controls. MCP-1 was elevated in uncomplicated influenza but not in IE. IL-4, IL-12, IL-17, IFN-γ, MIP-1β, and VEGF were not elevated